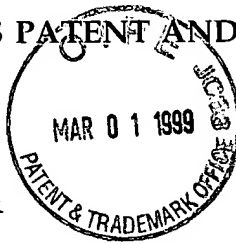


9200

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Patent No.: 5,057,540
Issued: October 15, 1991
In the Name of: Charlotte A. Kensil and Dante J. Marciani
For: Saponin Adjuvant
Docket No.: 106941.141



CobC
R

REVIEW

TRANSMITTAL LETTER

Assistant Commissioner for Patents
Washington, DC 20231

CERTIFICATE

MAR 16 1999

Sir:

OF CORRECTION

I hereby certify that this correspondence is being deposited with the United States Postal Service as First Class Mail in an envelope addressed to the Commissioner of Patents and Trademarks, Washington D.C. 20231.

on Feb. 23, 1999
(Date of Deposit)
Karen Kenney
Person Making Deposit
Km [Signature]
Signature

The following documents are submitted for filing:

1. Request for a Certificate of Correction Under 37 C.F.R. § 1.323;
2. A copy of the April, 1985 "VYDAC HPLC COLUMNS AND PACKING MATERIALS" catalog from The Separations Group;
3. Certificate of Correction; and
4. Return Postcard.

The Commissioner is authorized to debit the fee set forth in 37 C.F.R. § 1.20(a) of \$100.00 from Deposit Account No. 08-0219. No other fee is believed to be due, however, if one is required, the Commissioner is authorized to debit such fee from Deposit Account No. 08-0219.

Dated: February 23, 1999

Respectfully submitted,

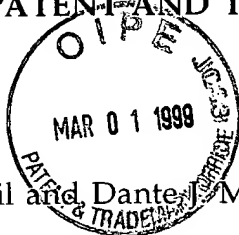
Colleen Superko

Hale and Dorr LLP
60 State Street
Boston, MA 02109
(617) 526-6000

Colleen Superko
Registration No. 39,850

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Patent No.: 5,057,540
Issued: October 15, 1991
In the Name of: Charlotte A. Kensil and Dante J. Marciani
For: Saponin Adjuvant
Docket No.: 106941.141



REQUEST FOR A CERTIFICATE OF CORRECTION
UNDER 37 C.F.R. § 1.323

Assistant Commissioner for Patents
Washington, DC 20231

Sir:

The attached Certificate of Correction is made under 37 C.F.R. § 1.323 and is respectfully requested to be issued under 35 U.S.C. § 255.

In this Request for a Certificate of Correction, it shows that mistakes occurred in good faith and do not involve such changes in the patent as would either constitute new matter or would require reexamination.

The Commissioner is authorized to debit the fee set forth in 37 C.F.R. § 1.20(a) of \$100.00 from Deposit Account No. 08-0219. No other fee is believed to be due, however, if one is required, the Commissioner is authorized to debit such fee from Deposit Account No. 08-0219.

This Request for a Certificate of Correction is to correct the pore size for the Vydac C₄ columns to 300 Å and to correct the pseudo-molecular ion mass of QA-21 to 1988.

I hereby certify that this correspondence is being deposited with the United States Postal Service as First Class Mail in an envelope addressed to: Commissioner of Patents and Trademarks, Washington D.C. 20231.

on Feb. 23, 1999
(Date of Deposit)

Karen Kenney
Person Making Deposit

[Signature]
Signature

U.S. Patent No. 5,057,540
Request for a Certificate of Correction

This request is being made for the following reasons:

The Vydac C₄ columns referenced in the specification and in the claims are commercially available reverse phase high pressure liquid chromatography ("HPLC") columns from The Separations Group, Hesperia, California. In addition, "Vydac" is a trademark of The Separations Group.

To support this Request for a Certificate of Correction, attached is a copy of the "VYDAC HPLC COLUMNS AND PACKING MATERIALS" catalog from The Separations Group, dated April, 1985. The catalog lists the complete line of HPLC columns that were commercially available at the time the underlying patent application was filed. On page 1 of the catalog is an index identifying the Vydac C₄ reverse phase HPLC columns as product "214 TP: Wide pore C₄ Reverse Phase" and indicating pages 8-9 of the catalog for a description. On page 8 of the catalog, the Vydac C₄ column is described as "[a] large pore (300 Å) spheroidal column packing material - ideal for large biomolecules - with an endcapped C-4 reverse phase." On page 9 of the catalog, the table shows that there are eleven different columns sold as Vydac C₄ columns, all with the same 300 Å pore size, but with different particle sizes, different column interior diameters ("i.d."), and different lengths.

There are two different kinds of Vydac C₄ columns referenced in the instant specification and one kind of Vydac C₄ column referenced in the claims. In the specification, a preparative Vydac C₄ column has a 5 µm particle size, 10 mm column i.d.

and 25 cm length. An analytical Vydac C₄ column having a 5 μ m particle size, 4.6 mm column i.d., and 25 cm length is the second type described in the specification. In the claims, reference is made only to the analytical Vydac C₄ column, that is a Vydac C₄ column having 5 μ m particle size, 4.6 mm column i.d., and 25 cm length.

Thus, it is clear that any Vydac C₄ column always has a 300 Å pore size. For identification purposes, then, the patent could have simply listed the Vydac C₄ columns as a 5 μ m particle size, 4.6 mm column i.d. (or 10 mm column i.d.) and 25 cm length. The 300 Å pore size is not a necessary characteristic to be listed as, by definition from the manufacturer, all Vydac C₄ columns have a 300 Å pore size.

In the specification and the claims, all references, except one, as noted below, erroneously give the pore size of the Vydac C₄ columns as 330 Å. As pointed out above, the correct pore size for the Vydac C₄ reverse phase HPLC columns is 300 Å.

The one reference to the correct pore size for the Vydac C₄ column is made in the patent specification at column 16, the sixth line under TABLE 4. Here, the HPLC conditions are provided as "Vydac [C₄], 5 μ m particle size, 300 Å pore size, .46 x 25 cm."

Another error occurred with regard to the pseudo-molecular ion mass of QA-21. The specification erroneously refers to the pseudo-molecular ion mass of QA-21 as 1980. The correct pseudo-molecular ion mass for QA-21 is 1988. The correct ion mass of QA-21 is found in the accompanying figures. Figure 8C, which is sheet 16 of 23 in the patent, provides the mass spectroscopy fast action bombardment of QA-21 and indicates,

U.S. Patent No. 5,057,540
Request for a Certificate of Correction

on the far right hand side, that the appropriate pseudo-molecular ion mass of QA-21 is 1988.

Hence, the errors in providing the pore size for the Vydac C₄ column as 300 Å and in providing the pseudo-molecular ion mass of QA-21 as 1980 were typographical errors. The errors were made in the first draft of the patent application, with the exceptions noted, and continued to be made throughout the prosecution of the application. The errors were made in good faith and without deceptive intent. Further, as shown above, correcting the pore size and the pseudo-molecular ion mass mistakes are of minor character that do not involve a change in the patent that would either constitute new matter or would require reexamination.

Although the 300 Å pore size was correctly given with TABLE 4 and the 1988 ion mass was correctly given in Figure 8C, the errors of the incorrect descriptions of a 330 Å pore size and of the 1980 ion mass were not noticed until it was first brought to the attention of the patent assignee in a European Opposition of the corresponding European Patent. The errors were made in good faith and without deceptive intent.

In conclusion, correcting the pore size from 330 Å to 300 Å and the pseudo-molecular mass of QA-21 from 1980 to 1988 are mistakes of minor character, that are not the fault of the Patent and Trademark Office, and a showing has been made that such mistakes occurred in good faith and without deceptive intent. Again, the corrections do



U.S. Patent No. 5,057,540
Request for a Certificate of Correction

not involve such changes in the patent as would either constitute new matter or would require reexamination.

The Commissioner is therefore respectfully requested to issue the attached Certificate of Correction.

Respectfully submitted,

A handwritten signature in cursive script that reads "Colleen Superko".

Colleen Superko
Registration No. 39,850

Dated: February 23, 1999

Hale and Dorr LLP
60 State Street
Boston, MA 02109
(617) 526-6000

/netuser6/karenb/baker/aquila/106941.141/certcorr.wpf

UNITED STATES PATENT AND TRADEMARK OFFICE
CERTIFICATE OF CORRECTION

PATENT NO.: 5,057,540
DATED: October 15, 1991
INVENTORS: Charlotte A. Kensil and Dante J. Marciani

It is certified that errors appear in the above-identified patent and that said Letters Patent is hereby corrected as shown below:

At column 4, line 53, "330 Å" should be -- 300 Å --.
At column 5, line 66, "330 Å" should be -- 300 Å --.
At column 6, line 2, "330 Å" should be -- 300 Å --.
At column 6, line 18, "330 Å" should be -- 300 Å --.
At column 6, line 22, "330 Å" should be -- 300 Å --.
At column 6, line 39, "330 Å" should be -- 300 Å --.
At column 6, line 43, "330 Å" should be -- 300 Å --.
At column 6, line 59, "330 Å" should be -- 300 Å --.
At column 6, line 62, "330" should be -- 300 --.
At column 9, line 43, "330 Å" should be -- 300 Å --.
At column 10, line 31, "330 Å" should be -- 300 Å --.
At column 12, line 56, "1980" should be -- 1988 --.
In claim 1, at column 22, line 63, "330 Å" should be -- 300 Å --.
In claim 2, at column 23, line 8, "330 Å" should be -- 300 Å --.
In claim 5, at column 23, line 33, "330 Å" should be -- 300 Å --.
In claim 8, at column 23, line 59, "330 Å" should be -- 300 Å --.
In claim 11, at column 24, line 25, "330 Å" should be -- 300 Å --.

MAILING ADDRESS OF SENDER
Colleen Superko
Hale and Dorr LLP
60 State Street
Boston, Massachusetts 02109

PATENT NO. 5,057,540



VYDA

HPLC COLUMNS AND PACKING MATERIALS

THE SEP/A/RA/TIONS GROUP

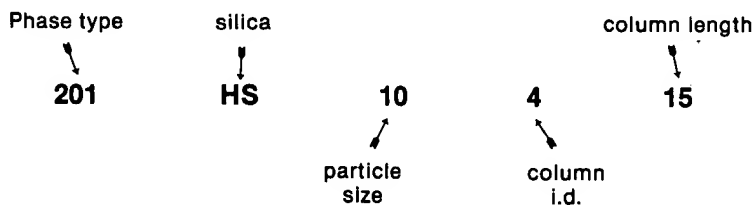
P. O. Box 867
Hesperia, California 92345
(619) 244-6107

APRIL 1985

About the covers...

Vydac High-Performance Liquid Chromatography columns and packing materials are manufactured in a quiet, beautiful location in the California high desert typified by the abundant Joshua trees unique to this location.

Vydac products are described by a catalog number such as the following:



This catalog number describes a column packed with reverse phase 201 on 10 micron HS silica, 4.6 mm in diameter (only the integer is used) and fifteen cm in length (catalog numbers for 25 cm columns omit the column length as this is standard). Catalog numbers for bulk materials have a 'B' after the silica type and no column dimensions: an example is '201HSB10' to describe bulk 201 reverse phase on 10 micron HS silica.

Vydac catalog numbers designate the bonded phase as:

100 series	Unbonded silica
200 series	Reverse phase
300 series	Anion exchange
400 series	Cation exchange
500 series	Cyano polar phase
600 series	Amino polar phase

Vydac columns feature high-quality Valco inverted endfittings.

The Separations Group is the leading manufacturer of high purity HPLC spheroidal silica in the world today with fourteen years experience providing quality HPLC columns to the analytical community. Our **Vydac** HPLC columns are *unique*—just like our California high desert location. We manufacture our own **high purity silicas**, carefully size them into narrow particle size ranges and chemically bond various chromatographic phases. Each batch of material is carefully tested for selectivity and packing efficiency after which columns are packed by proprietary methods. Every column is individually tested to assure the highest quality final product. These steps combine to produce exceptional columns with unique separation characteristics. **Vydac** columns and packing materials stand out from the crowd and are frequently the choice of knowledgeable scientists in their respective application fields.

Vydac is a trademark of The Separations Group

INDEX	
Product	Page No.
Silica substrates	2-3
201 HS: High Capacity Reverse Phase	4-5
201 TP: Medium capacity Reverse Phase	6-7
214 TP: Wide pore C ₁₈ Reverse Phase	8-9
218 TP: Wide pore C ₁₈ Reverse Phase	10-11
219 TP: Wide pore phenyl Reverse Phase	12-13
Preparative HPLC	14-15
Ion chromatography	16-17
Ion exchange	18
Solid Core (Pellicular)/Pre-column kits	19
Selected Bibliography	20-21

For analytical to preparative separations . . .

Vydac columns are available in three diameters: analytical (4.6 mm), semi-preparative (10 mm) and preparative (22 mm). Bulk packing material is available in 10 micron, 15-20 micron and 20-30 micron particle sizes of the same silica substrate and using the same bonding chemistry, permitting direct scale-up of analytical separations.

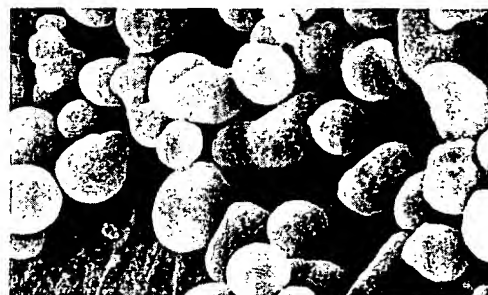
The beginning...

High Purity Silica

The Separations Group manufactures two types of high purity silica for high-performance liquid chromatography:

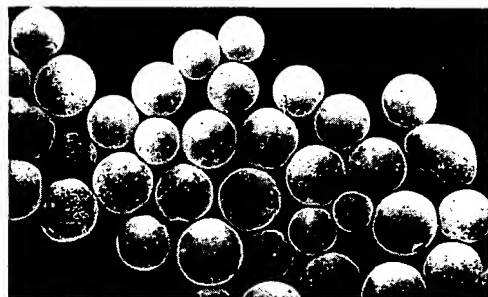
Vydac TP Silica

TP silica is spheroidal, with 300 angstrom pores, a low surface area of 80 sq. m. per gram and a pore volume of .6 cc/gram. The wide pores in TP silica make it ideal for the separation of large bio-molecules. Its low surface area results in a medium capacity reverse phase, making it an excellent material for the chromatography of very hydrophobic small molecules.



Vydac HS Silica

HS silica is spherical, with 80 angstrom pores, a surface area of 500 sq. m. per gm and a pore volume of .8 cc/gram. The high surface area of HS silica results in a high loading of bonded phase making this material particularly good for the separation of relatively hydrophilic compounds.



Both TP and HS silica are physically rugged and offer stable, reproducible columns with moderate backpressure even with small particles. The Separations Group has developed a family of quality HPLC separation products based on these high purity silicas. They are excellent substrates to which many chromatographic phases may be bonded. The combination of high purity silica and special bonding techniques yield unique separation materials.

The manufacturing process for Vydac TP and HS silica yields:

- *High purity silica, low in metal and sulphate content and free from extraneous chromatographic effects*
- *Rigid, physically rugged, spheroidal particles that do not fracture in the column thus reducing the backpressure and lengthening the lifetime of High-Performance Liquid Chromatography columns*
- *Reproducible materials that give the same separations column after column. Careful quality control assures the chromatographer of reliable results.*

Vydac unbonded TP and HS silicas are available in columns and bulk:

COLUMNS:

Catalog No.	Particle Size	Column I.d.	Length	Price
101HS54	5 micron	4.6 mm	25 cm	\$ 250
101HS104	10 micron	4.6 mm	25 cm	209
101TP54	5 micron	4.6 mm	25 cm	250
101TP104	10 micron	4.6 mm	25 cm	209

BULK:

Catalog No.	Particle Size	Price / gram			
		10 gm	100 gm	500 gm	1 kilogram
101HSB10	10 micron	13.20	10.80	8.90	8.60
101HSB1520	15-20 micron	---	2.50	2.50	1.75
101HSB2030	20-30 micron	---	2.00	1.50	1.30
101TPB10	10 micron	13.20	10.80	8.90	8.60
101TPB1520	15-20 micron	---	2.50	2.50	1.75
101TPB2030	20-30 micron	---	2.00	1.50	1.30

Bulk packing materials are available in larger quantities and other particle size ranges on special request. Ask for a quotation on price, delivery and minimum order quantity.

Vydac 201 HS Reverse Phase

201 HS is a high capacity reverse phase material. An eighteen carbon chain is chemically bonded to the high surface area HS silica which is then endcapped with trimethylsilane. 201HS columns and packing materials feature:

- A high surface area, spherical silica
- A high loading of the bonded phase resulting in a high capacity endcapped, C-18 reverse phase material
- Excellent separations for relatively polar compounds such as the B vitamins, organic acids and biological bases and nucleosides

Separation of B Vitamins

CONDITIONS

Solvent #1: 0.1M KOAc adj. to pH 4.7
with formic acid

Solvent #2: 50% acetonitrile, 50 % water

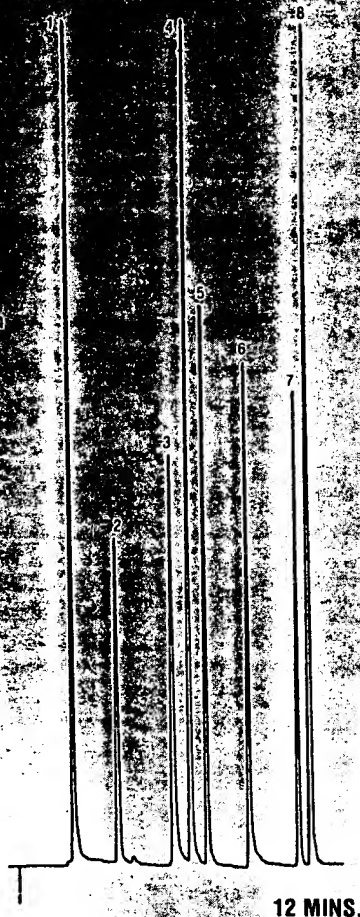
Start: 95% Solvent #1, 5% Solvent #2
Linear gradient programmed
over 15 min. to 100% Solvent #2

Flowrate: 1.5 ml/min

Detector: UV @ 254 nm.

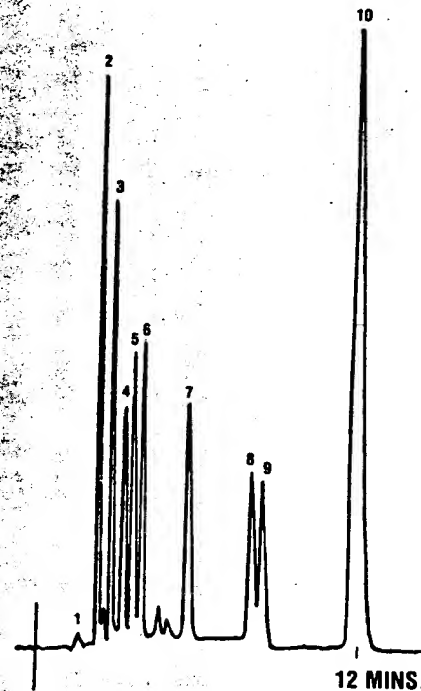
PEAK IDENTITY

1. Vitamin C - Ascorbic Acid
2. Nicotinic Acid - Niacin
3. Vitamin B₆ - Pyridoxine
4. Vitamin B₁ - Thiamine
5. Vitamin B₃ - Nicotinamide
6. Vitamin M - Folic Acid
7. Vitamin B₁₂ - Cyanocobalamin
8. Vitamin B₂ - Riboflavin



12 MINS.

Separation of Organic Acids



12 MINS.

CONDITIONS

Solvent: .01M Triethylamine
adj. to pH 2.0
Phosphoric Acid

Flowrate: 1.5 ml/min

Detector: UV @ 220 nm.

PEAK IDENTITY

- | | |
|-------------------|-------------------|
| 1. Solvent front | 6. Acetic Acid |
| 2. Glyoxalic Acid | 7. Citric Acid |
| 3. Tartaric Acid | 8. Succinic Acid |
| 4. Malic Acid | 9. Propionic Acid |
| 5. Lactic Acid | 10. Maleic Acid |

Best Available Copy

ANALYSIS OF NUCLEOSIDES AND BASES

PEAK IDENTITY

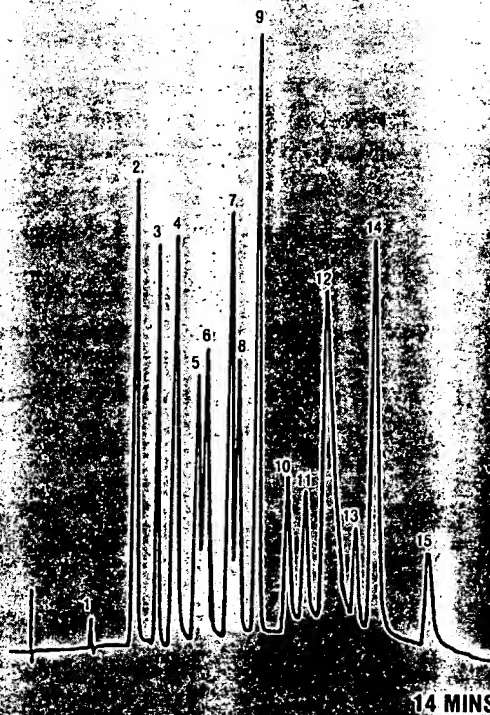
1. Solvent
2. Cytosine
3. Uracil
4. Cytidine
5. 5-methyl cytosine
6. Uridine
7. Hypoxanthine
8. Guanine
9. Thymine
10. Inosine
11. Guanosine
12. Xanthine
13. Thymidine
14. Adenine
15. Adenosine

CONDITIONS

Eluent: A: .01 M KOAc pH 5.0

B: 25:75 ACN:H₂O

Gradient: 5 to 100% B in 30 min



Vydac 201 HS is available in pre-packed columns and in bulk:

COLUMNS:

Catalog No.	Particle Size	Column I.d.	Length	Price
ANALYTICAL				
201HS5415	5 micron	4.6 mm	15 cm	\$ 300
201HS54	5 micron	4.6 mm	25 cm	325
201HS10415	10 micron	4.6 mm	15 cm	225
201HS104	10 micron	4.6 mm	25 cm	248
PREPARATIVE				
201HS510	5 micron	10 mm	25 cm	800
201HS1010	10 micron	10 mm	25 cm	600
201HS1022	10 micron	22 mm	25 cm	1600

BULK:

Catalog No.	Particle Size	Price / gram (\$)			
		10 gm	100 gm	500 gm	1 kilogram
201HSB10	10 micron	19.30	15.00	12.00	11.00
201HSB1520	15-20 micron	---	3.00	3.00	2.00
201HSB2030	20-30 micron	---	2.50	2.00	1.50

Bulk packing materials are available in larger quantities and other particle size ranges on special request. Ask for a quotation on price, delivery and minimum order quantity.

Vydac 201 TP Reverse Phase

201 TP is a medium capacity reverse phase material. An eighteen carbon chain is chemically bonded to the low surface area TP silica resulting in a medium level phase loading. 201 TP columns and packing materials feature:

- A low surface area, spheroidal silica
- A moderate loading of bonded phase resulting in a medium capacity non-encapped, C-18 reverse phase material
- Excellent separations for very non-polar compounds such as polynuclear aromatic hydrocarbons, aflatoxins and alpha and beta carotenes.

ANALYSIS OF POLYAROMATIC HYDROCARBONS

CONDITIONS

Gradient:

Solvent #1: H₂O

Solvent #2: Acetonitrile

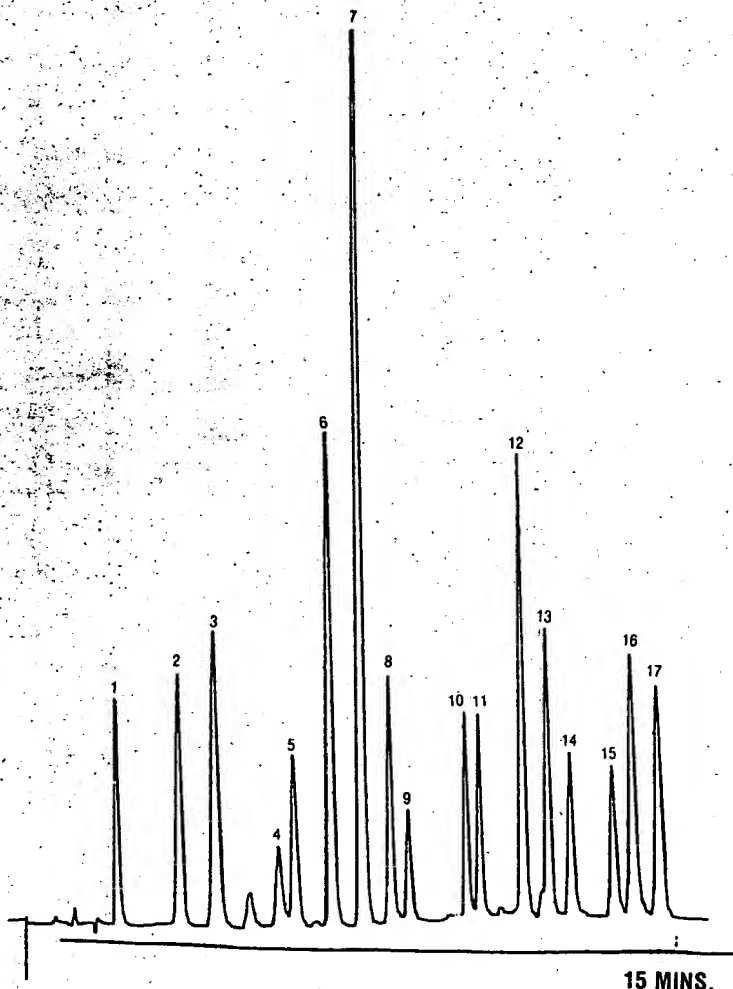
Start 50% solvent #2 for 3 minutes then run Linear Gradient programmed over 7 minutes to 100% solvent #2.

Flowrate: 1.5 ml/min

Detector: UV @ 254 nm.

PEAK IDENTITY

1. Benzene
2. Naphthalene
3. Acenaphthylene
4. Acenaphthene
5. Fluorene
6. Phenanthrene
7. Anthracene
8. Fluoranthene
9. Pyrene
10. Benz (a) anthracene
11. Chrysene
12. Benzo (b) fluoranthene
13. Benzo (k) fluoranthene
14. Benz (a) pyrene
15. Dibenzo (a, h) anthracene
16. Benzo (g, h, i) perylene
17. Indeno (1, 2, 3, c, d) pyrene



ALPHA AND BETA CAROTENES

Eluent: 90% Acetonitrile/10% Chloroform

Flowrate: 1.5 ml/min

Detector: UV @ 254 nm.

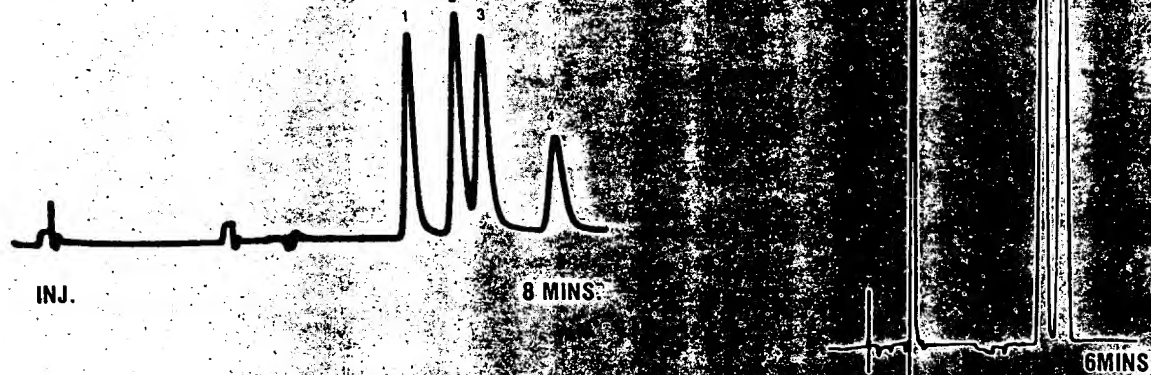
PEAK IDENTITY

1. Alpha-Carotene

2. Beta-Carotene

AFLATOXINS

Eluent: 100:20:20

H₂O:ACN:MeOH1. Aflatoxin G₂2. Aflatoxin G₁3. Aflatoxin B₂4. Aflatoxin B₁

Vydac 201TP is available in pre-packed columns and in bulk:

COLUMNS:

Catalog No.	Particle Size	Column i.d.	Length	Price
ANALYTICAL				
201TP5415	5 micron	4.6 mm	15 cm	\$ 300
201TP54	5 micron	4.6 mm	25 cm	325
201TP10415	10 micron	4.6 mm	15 cm	225
201TP104	10 micron	4.6 mm	25 cm	248
PREPARATIVE				
201TP510	5 micron	10 mm	25 cm	800
201TP1010	10 micron	10 mm	25 cm	600
201TP1022	10 micron	22 mm	25 cm	1600

BULK:

Catalog No.	Particle Size	Price / gram (\$)			
		10 gm	100 gm	500 gm	1 kilogram
201TP810	10 micron	19.30	15.00	12.00	11.00
201TP81520	15-20 micron	---	3.00	3.00	2.00
201TP82030	20-30 micron	---	2.50	2.00	1.50

Bulk packing materials are available in larger quantities or other particle size ranges on special request. Ask for a quotation on price, delivery and minimum order quantity.

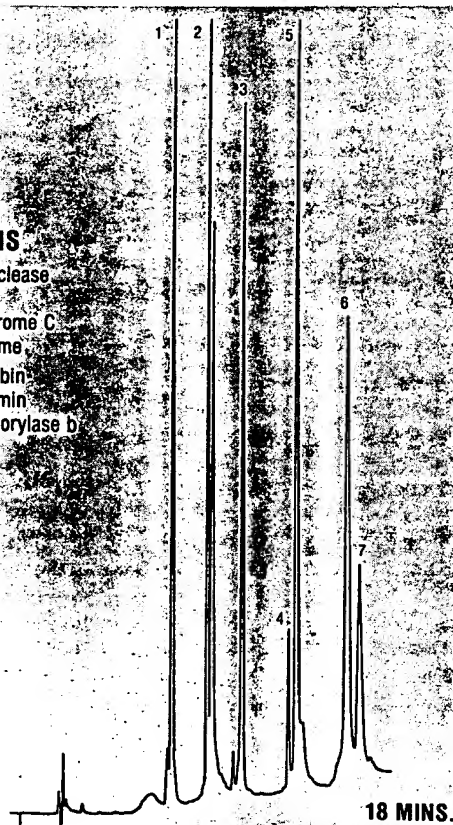
Vydac 214 TP Reverse Phase

214 TP is a short chain reverse phase material consisting of a four carbon chain chemically bonded to low surface area TP silica which is then endcapped with trimethylsilane to prevent adsorption of polar compounds. 214 TP columns and packing materials feature:

- **A premium column packing material—the choice of many bioscientists for their most demanding protein and peptide separations**
- **A large pore (300 Å) spheroidal column packing material—ideal for large biomolecules—with an endcapped C-4 reverse phase**
- **High purity silica with few free silanol groups resulting in good recovery for most proteins and peptides**
- **Excellent separations of peptides and proteins.**

PROTEINS

1. Ribonuclease
2. Insulin
3. Cytochrome C
4. Lysozyme
5. Myoglobin
6. Ovalbumin
7. Phosphorylase b

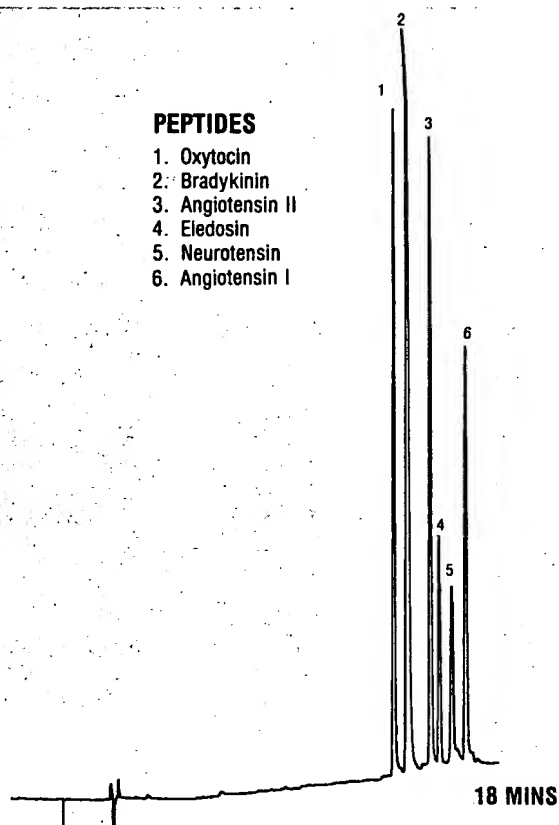


Solvent a: .1% TFA in H₂O
 b: .1% TFA in 95/5 ACN/H₂O
 Gradient: 25% B to 100% B over 30 min
 Flowrate: 1.5 ml/min

CONDITIONS

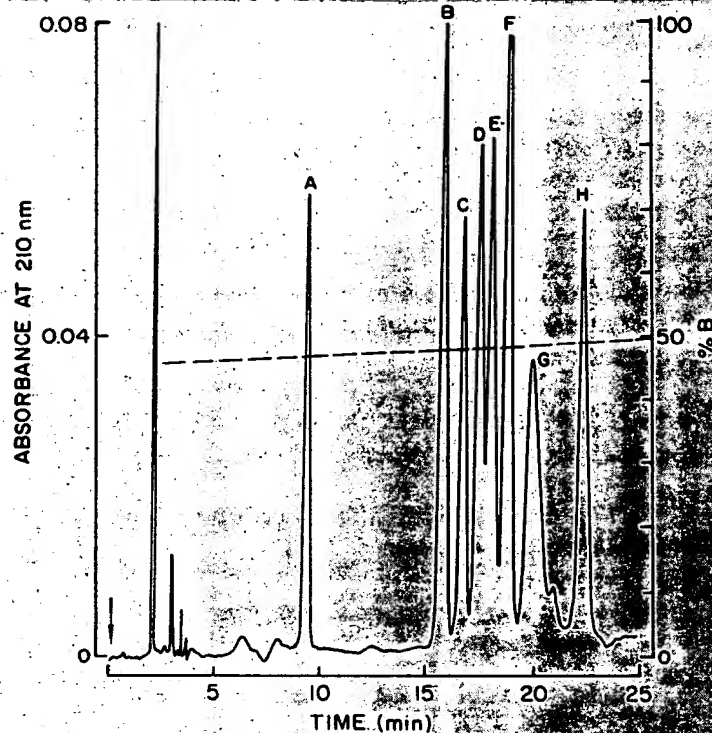
PEPTIDES

1. Oxytocin
2. Bradykinin
3. Angiotensin II
4. Eledosin
5. Neurotensin
6. Angiotensin I



Solvent a: .1% TFA in H₂O
 b: .1% TFA in 30/70 H₂O/ACN
 Gradient: 5% B to 100% B over 30 min
 Flowrate: 1.5 ml/min

SEPARATION OF INSULINS



Insulins: A) chicken, B) bovine, C) ovine, D) rabbit,
E) human, F) porcine, G) rat I, H) rat II

Eluents: A: .1% TFA in H₂O B: .1% TFA in ACN:H₂O (60:40)

Gradient: 45% to 50% B in 25 min

reprinted by permission of journal and authors from J. Rivier and R. McClintock, J. Chrom 268, 112 (1983)

Vydac 214 TP is available in pre-packed columns and in bulk:

COLUMNS:

Catalog No.	Particle Size	Column I.d.	Length	Price
ANALYTICAL				
214TP5415	5 micron	4.6 mm	15 cm	\$ 300
214TP54	5 micron	4.6 mm	25 cm	325
214TP10415	10 micron	4.6 mm	25 cm	225
214TP104	10 micron	4.6 mm	25 cm	248

PREPARATIVE

214TP510	5 micron	10 mm	25 cm	800
214 TP1010	10 micron	10 mm	25 cm	600
214TP1022	10 micron	22 mm	25 cm	1600
214TP152022	15-20 micron	22 mm	25 cm	995

BULK:

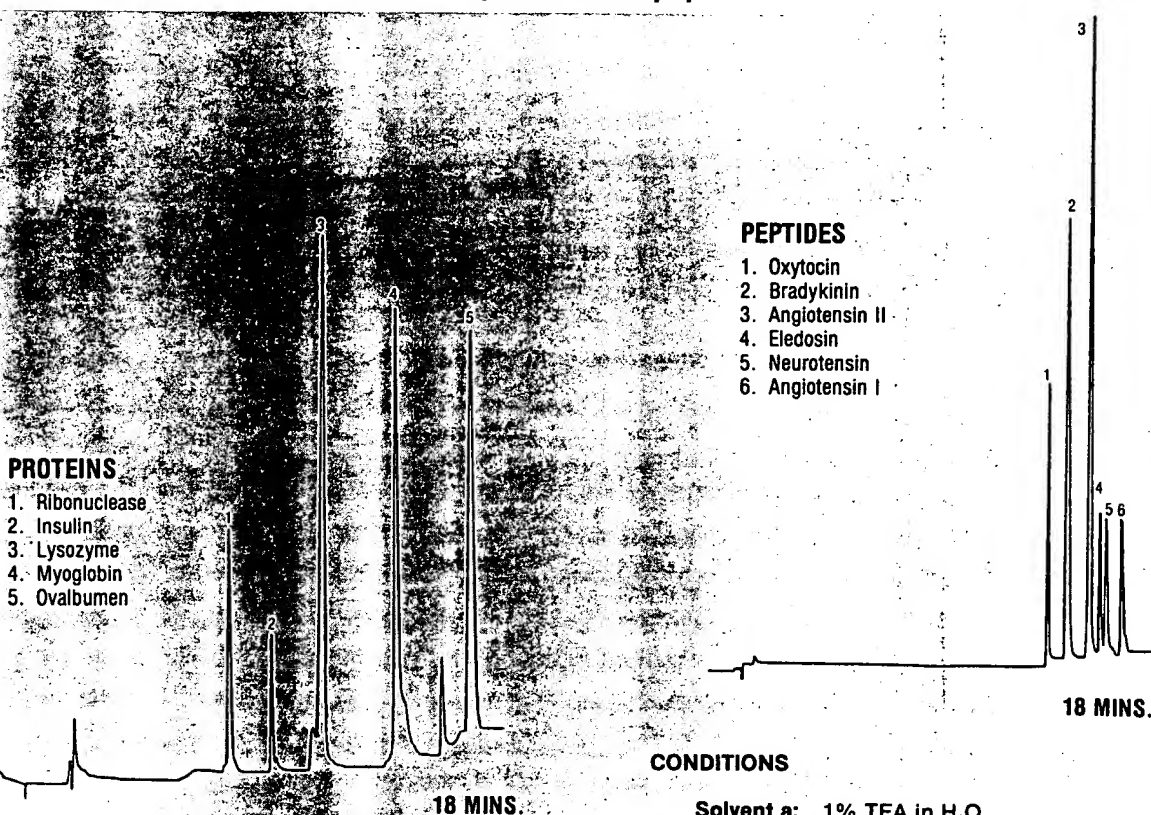
Catalog No.	Particle Size	Price / gram (\$)			
		10 gm	100 gm	500 gm	1 kilogram
214TPB10	10 micron	19.30	15.00	12.00	11.00
214TPB1520	15-20 micron	---	3.00	3.00	2.00
214TPB2030	20-30 micron	---	2.50	2.00	1.50

Bulk packing materials are available in larger quantities or other particle size ranges on special request. Ask for a quotation on price, delivery and minimum order quantity.

Vydac 218 TP Reverse Phase

218 TP is a long chain reverse phase material consisting of an eighteen carbon chain chemically bonded to low surface area TP silica which is then endcapped with trimethylsilane to prevent adsorption of polar compounds. 218 TP columns and packing materials feature:

- **A premium column packing material—together with its companion, 214 TP, the choice of many bioscientists for their most demanding protein and peptide separations**
- **A large pore (300Å) spheroidal column packing material—ideal for large biomolecules—with an endcapped C-18 reverse phase**
- **High purity silica with few free silanol groups resulting in good recovery for most proteins and peptides**
- **Excellent separations of proteins and peptides**



CONDITIONS

Solvent a: .1% TFA in H₂O
 b: .1% TFA in 30/70 H₂O/ACN
 Gradient: 5% to 100% B over 30 min
 Flowrate: 1.5 ml/min

CONDITIONS

Solvent a: .1% TFA in H₂O
 b: .1% TFA in 95/5 ACN/H₂O
 Gradient: 25% B to 100% B over 30 min
 Flowrate: 1.5 ml/min

TRYPTIC DIGEST OF CYTOCHROME c

CONDITIONS

Solvent Gradient

Solvent #1: 0.1% TFA in water

Solvent #2 0.1% TFA in 70% acetonitrile/
30% water

Linear Gradient programmed over 2 hours
from 100% of solvent #1 to 100% solvent #2

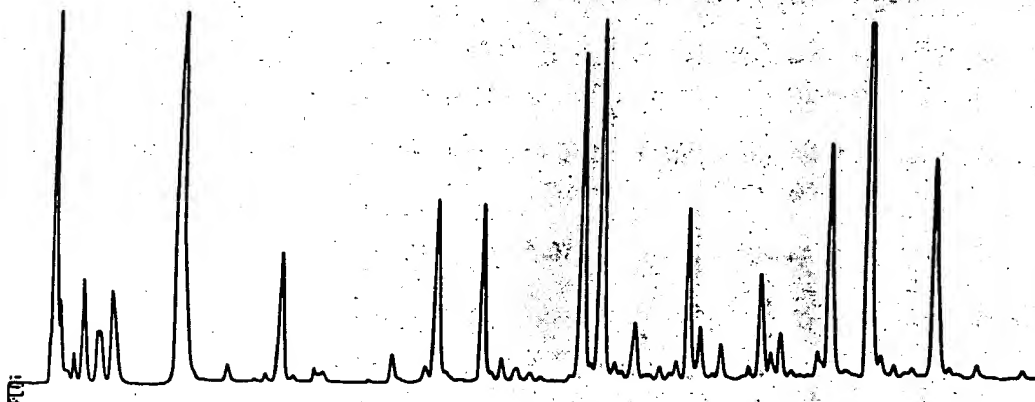
Flowrate: 1 ml/min

Detector: UV@ 214 nm

Cytochrome C Tryptic Digest-30 micrograms

Courtesy of:

Dr. Brian Clark
City of Hope Research Institute
Division of Immunology
Duarte, CA 91010



Vydac 218 TP is available in pre-packed columns and in bulk:

COLUMNS:

Catalog No.	Particle Size	Column I.d.	Length	Price
ANALYTICAL				
218TP5415	5 micron	4.6 mm	15 cm	\$ 300
218TP54	5 micron	4.6 mm	25 cm	325
218TP10415	10 micron	4.6 mm	15 cm	225
218TP104	10 micron	4.6 mm	25 cm	248
PREPARATIVE				
218TP510	5 micron	10 mm	25 cm	800
218TP1010	10 micron	10 mm	25 cm	600
218TP1022	10 micron	22 mm	25 cm	1600
218TP152022	15-20 micron	22 mm	25 cm	995

BULK:

Catalog No.	Particle Size	Price / gram (\$)			
		10 gm	100 gm	500 gm	1 kilogram
218TPB10	10 micron	19.30	15.00	12.00	11.00
218TPB1520	15-20 micron	---	3.00	3.00	2.00
218TPB2030	20-30 micron	---	2.50	2.00	1.50

Bulk packing materials are available in larger quantities or other particle size ranges on special request. Ask for a quotation on price, delivery and minimum order quantity.

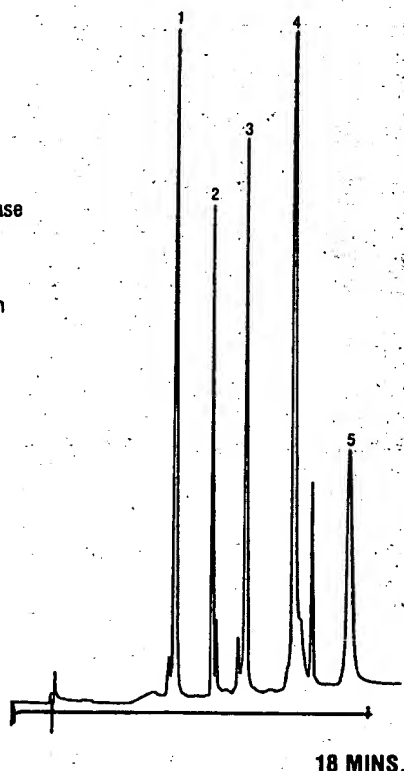
Vydac 219 TP Reverse Phase

219 TP is an aromatic reverse phase material consisting of a diphenyl group chemically bonded to low surface area TP silica which is then endcapped with trimethylsilane to prevent adsorption of polar compounds. 219 TP columns and packing material feature:

- **An alternative selectivity for proteins and peptides by means of the aromatic diphenyl reverse phase**
- **A large pore (300 Å) spheroidal column packing material—ideal for large biomolecules**
- **High purity silica with few free silanol groups resulting in good recovery for most proteins and peptides**
- **Excellent separations of proteins and peptides**

PROTEINS

1. Ribonuclease
2. Insulin
3. Lysozyme
4. Myoglobin
5. Ovalbumin

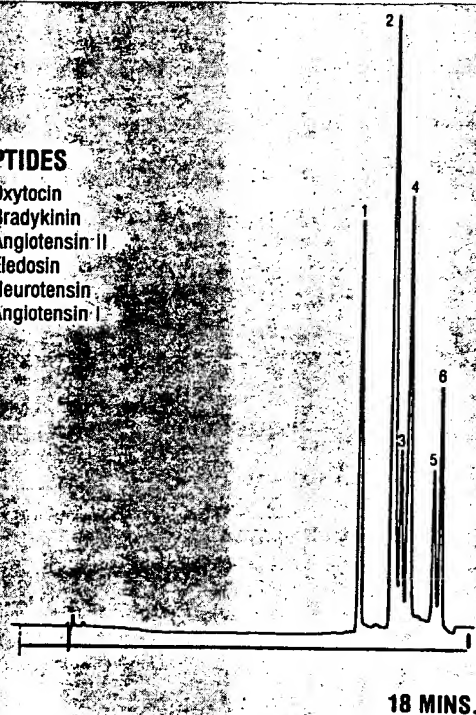


CONDITIONS

Solvent a: .1% TFA in H₂O
 b: .1% TFA in 95/5 ACN/H₂O
 Gradient: 25% B to 100% B over 30 min
 Flowrate: 1.5 ml/min

PEPTIDES

1. Oxytocin
2. Bradykinin
3. Angiotensin II
4. Eledosin
5. Neurotensin
6. Angiotensin I

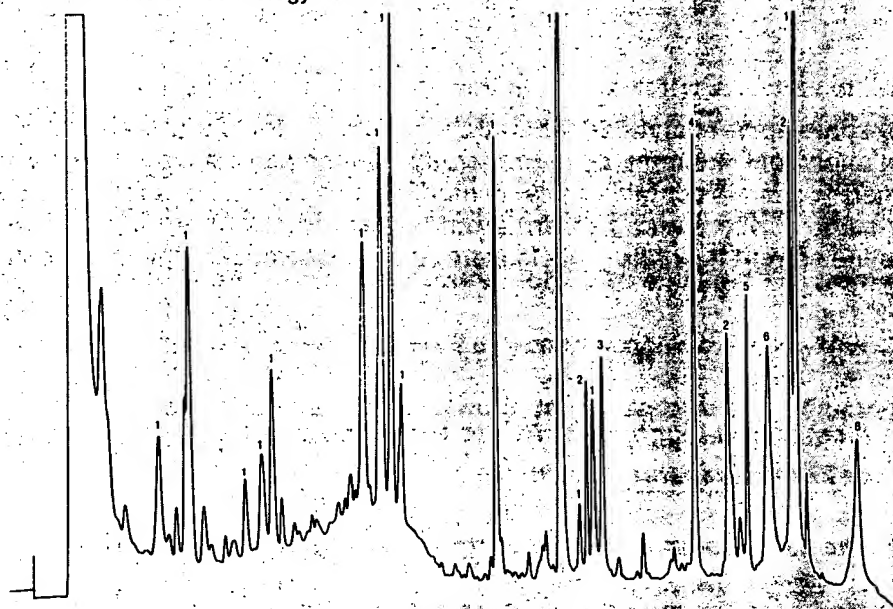


CONDITIONS

Solvent a: .1% TFA in H₂O
 b: .1% TFA in 30/70 H₂O/ACN
 Gradient: 5% to 100% B over 30
 Flowrate: 1.5 ml/min

Unpublished Data

Courtesy of: Dr. Paul Tempst
Division of Biology
Cal Institute of Technology



Peak Identity

- 1: Horse cytochrome c peptides
- 2: Whale myoglobin CNBr fragments
- 3: Ribonuclease
- 4: Cytochrome c
- 5: Lysozyme
- 6: BSA
- 7: Myoglobin
- 8: Ovalbumin

Protein/Peptide Mixture

Solvent a: 0.1% TFA
Solvent b: 0.1% TFA in 70:30 ACN:H₂O
Gradient: 0-50% 50 min
50-70% 10 min
70-100% 7.5 min

Vydac 219 TP is available in pre-packed columns and in bulk:

COLUMNS:

Catalog No.	Particle Size	Column i.d.	Length	Price
ANALYTICAL				
219TP5415	5micron	4.6 mm	15 cm	\$ 300
219TP54	5 micron	4.6 mm	25 cm	325
219TP10415	10 micron	4.6 mm	15 cm	225
219TP104	10 micron	4.6 mm	25 cm	248
PREPARATIVE				
219TP510	5 micron	10 mm	25 cm	800
219TP1010	10 micron	10 mm	25 cm	600
219TP1022	10 micron	22 mm	25 cm	1600
219TP152022	15-20 micron	22 mm	25 cm	995

BULK:

Catalog No.	Particle Size	Price / gram (\$)			
		10 gm	100 gm	500 gm	1 kilogram
219TPB10	10 micron	19.30	15.00	12.00	11.00
219TPB1520	15-20 micron	---	3.00	3.00	2.00
219TPB2030	20-30 micron	---	2.50	2.00	1.50

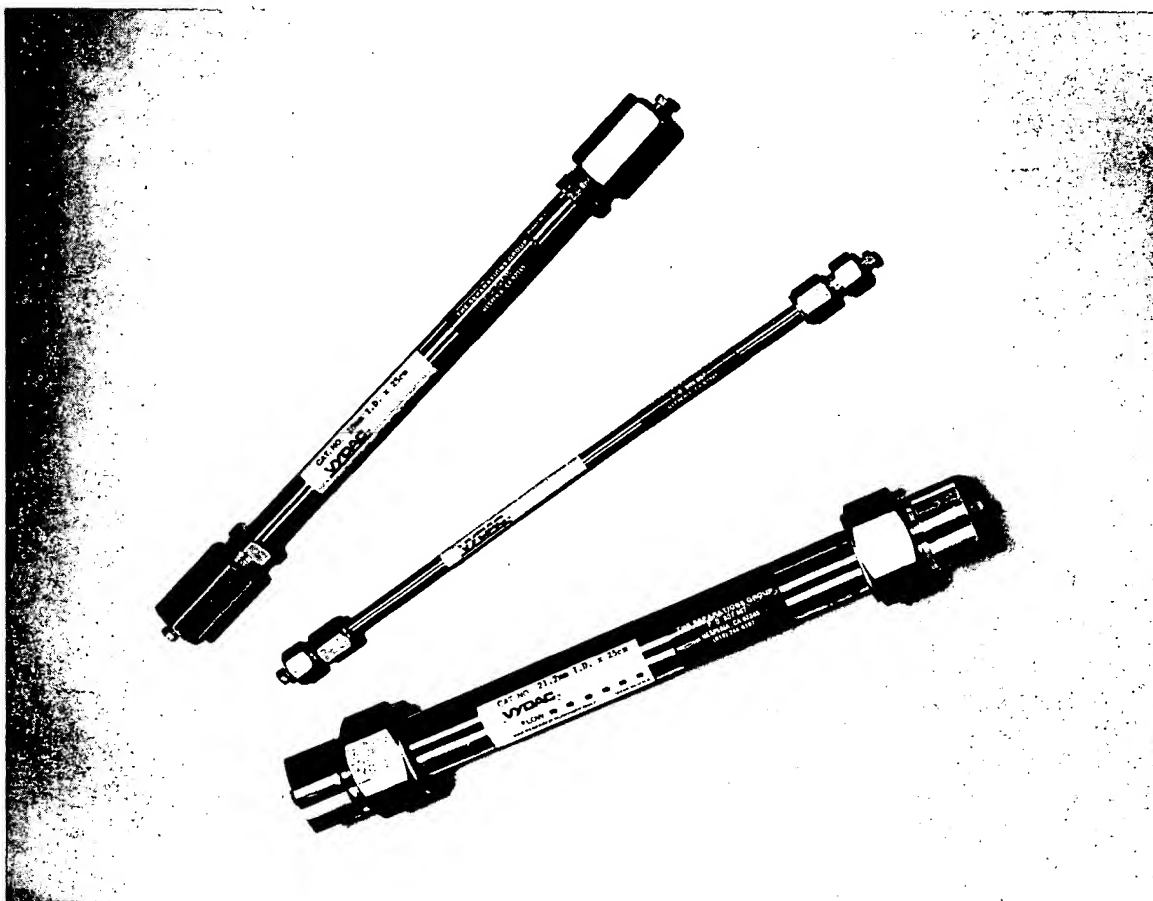
Bulk packing materials are available in larger quantities or other particle size ranges on special request.
Ask for a quotation on price, delivery and minimum order quantity.

Preparative HPLC Separations

For simple scale-up of analytical separations **Vydac** columns are available in three diameters: analytical (4.6 mm), semi-preparative (10 mm) and preparative (22 mm). Large diameter columns contain the same material as analytical columns and permit use of the same solvents and gradient profile. The only parameter that must be changed is the solvent flow rate. This is increased to accomodate the larger column volume.

Vydac preparative columns feature:

- ***Increased throughput of five to twenty times with 10 mm semi-preparative or 22 mm preparative columns***
- ***The same column packing materials are used in analytical columns***
- ***Moderate backpressure at normal operating flow rates***
- ***Direct scaleup of analytical separations by retaining both the solvents and gradient profile used with an analytical column while increasing the flow rate***



See individual product ordering information for the catalog numbers and prices of available semi-preparative and preparative columns.

Vydac column packing materials are offered in a range of particle sizes to allow simple scale-up of separations achieved on analytical or semi-preparative columns. **Vydac** bulk packing materials feature:

- **Three particle size ranges for maximum flexibility: a narrow distribution ten micron material, a 15-20 micron material and a 20-30 micron material**
- **The same silica substrate except for particle size and the same bonded phases as small micron analytical column packing materials**
- **Simple column packing procedures for 15-20 and 20-30 micron materials**
- **Low packpressure requirements at normal operating flow rates**
- **Little loss in resolution scaling up to larger particle size column packing materials.**

COMPARISON OF PROTEIN SEPARATION ON DIFFERENT PARTICLE SIZE PACKING MATERIALS

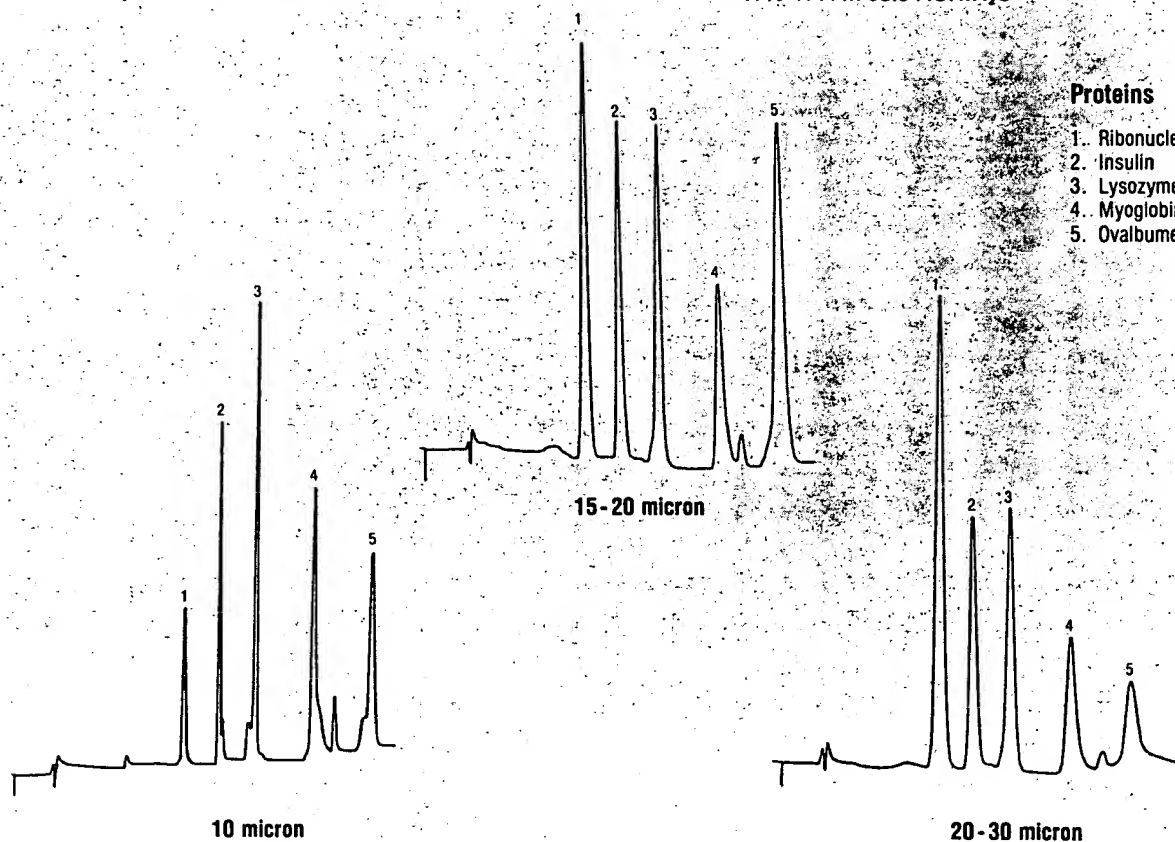
CONDITIONS

Material: Vydac 218 TP
Sample: Protein Standard

Eluent: .1% TFA in 25:75 ACN:H₂O to
.1% TFA in 95:5 ACN:H₂O

Proteins

1. Ribonuclease
2. Insulin
3. Lysozyme
4. Myoglobin
5. Ovalbumin



See individual products for prices, catalog number, and quantity discounts on bulk packing material.

Vydac Ion Chromatography Columns

Vydac columns for non-suppressed ion chromatography set a new standard of performance for single column ion analysis by HPLC. Vydac 300IC and 400IC columns feature a small particle, low surface area silica that has been protected with a proprietary chemical treatment developed by the Separations Group permitting these columns to be used above pH 7. These low capacity ion exchange columns have been specifically designed for the separation of ions and feature:

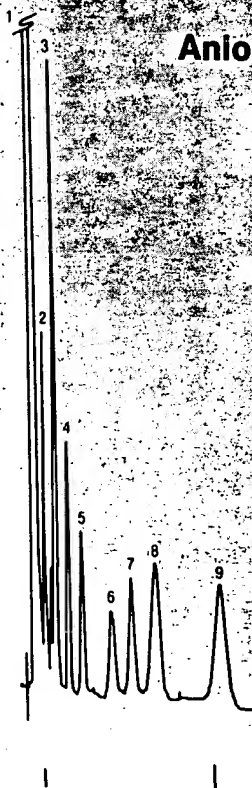
- **Single column technology allowing use of standard HPLC components**
- **A highly efficient silica substrate**
- **Use of eluents as high as pH 9 with no column degradation**
- **Rapid analysis (2-5 minutes) with excellent resolution**
- **High sensitivity (typically sub-ppm detectability for most ions)**
- **Also available is the 302IC column for anion analysis. Although not stable in basic eluents, this column remains a standard in single column ion analysis.**

Anion Analysis

Column: Vydac 300IC405
Eluent: 1.5 mM phthalate
pH 8.9

Peak Identity

1. Solvent
2. F^- 5 ppm
3. CO_3^{2-} dissolved
4. Cl^- 1 ppm
5. NO_2^- 1.5 ppm
6. Br^- 3 ppm
7. NO_3^- 2.5 ppm
8. HPO_4^{2-} 3 ppm
9. SO_4^{2-} 3 ppm



4 min.

Cation Analysis

Column: Vydac 400IC405
Eluent: 2.5 mM HNO_3

Peak Identity

1. Li^+
2. Na^+
3. NH_4^+
4. K^+
5. Rb^+
6. Cs^+



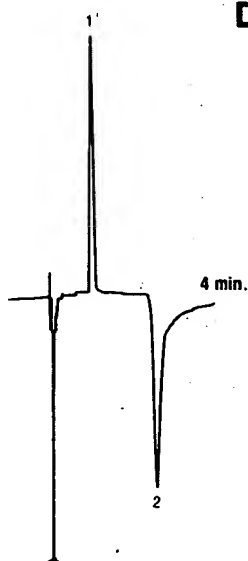
6 min.

Dichromate

Column: Vydac 300IC405
Eluent: 3 mM phthalic acid
pH 8.0

Peak Identity

1. Sulphate
2. Dichromate



Fluorophosphate

Column: Vydac 300IC405
Eluent: 2.5 mM phthalic acid
pH 8.6

Peak Identity

1. Fluoride
2. Phosphate
3. Fluorophosphate

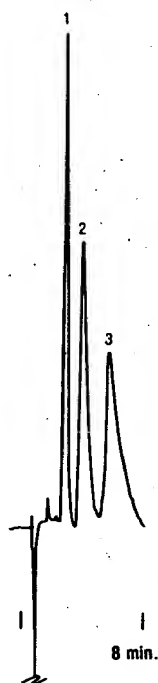


Amines

Column: Vydac 400IC405
Eluent: .01 M H₃PO₄
+ 10% MeOH

Peak Identity

1. Methamine
2. Dimethylamine
3. Trimethylamine

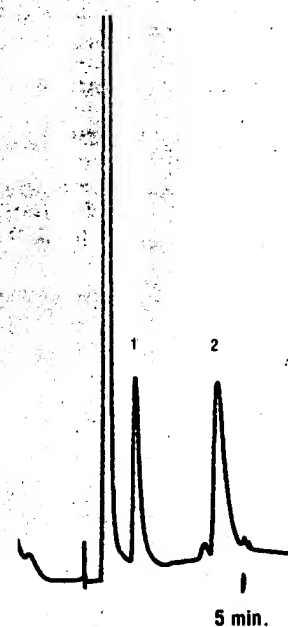


Organic Acids

Column: Vydac 302IC4.6
Eluent: 1 mM Phthalic Acid
pH 4.6

Peak Identity

1. Acetate
2. Formate



Vydac Ion Chromatography materials are available only in pre-packed columns.

Catalog No.	Type	Column i.d.	Length	Price
300IC405	Anion	4.6 mm	5 cm	\$ 350
302IC4.6	Anion	4.6 mm	25 cm	240
400IC405	Cation	4.6 mm	5 cm	350

Vydac Ion Exchange Columns and Packing Materials

Vydac microparticulate ion exchange materials are available with both anion and cation exchange groups. These are chemically bonded to ten micron low surface area TP silica. Vydac microparticulate ion exchange materials include:

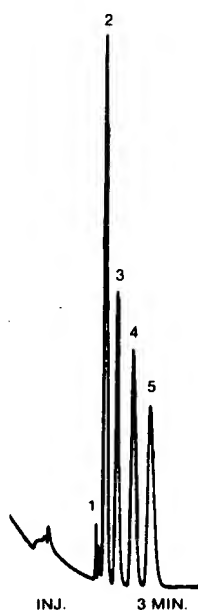
- **301 TP**, a strong anion exchange material with a quaternary amine functional group for the analysis of nucleotides and other organic acids
- **401 TP**, a strong cation exchange material with a sulphonic acid functional group for analysis of amines, purines, pyrimidines and other organic bases.

301 TP

NUCLEOTIDES

1. Solvent
2. CMP
3. AMP
4. UMP
5. GMP

Eluent:
.05 M NaH_2PO_4
pH 2.79

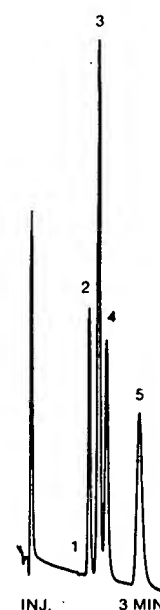


401TP

NUCLEOSIDES

1. Solvent
2. Uracil
3. Cytosine
4. Hypoxanthine
5. 5-Methyl cytosine

Eluent:
.1 N NH_4OAc
pH 5.27



301 TP and 401 TP ion exchange materials are available in pre-packed columns and in bulk:

COLUMNS:

Catalog No.	Type	Column I.d.	Length	Price
301TP104	Anion	4.6 mm	25 cm	\$ 264
401TP104	Cation	4.6 mm	25 cm	264

BULK:

Catalog No.	Type	Price (10 gm)
301TPB10	Anion	\$218.00
401TPB10	Cation	218.00

Note: request quotation for larger quantities of bulk.

Vydac Solid Core Materials and Pre-column Kits

Vydac SC (Solid Core) is a unique separation material made by chemically coating a solid glass support with silica to which various phases are chemically bonded.

Vydac SC has a surface area of 8 sq. m./gram and a particle size distribution centered at 30 microns. Generally used for pre-columns, **Vydac SC** materials feature:

- *Free flowing, high density material for easy packing of columns*
- *Thermally and hydrolytically stable material for reliable, extended use*
- *Available in bulk and in pre-column kits*

BULK SOLID CORE

Catalog No.	Material	Price (25 grams)
101SC	Silica adsorbent	\$ 129.00
201SC	Reverse phase (C ₁₈)	184.00
301SC	Anion exchange	216.50
401SC	Cation exchange	216.50
501SC	Polar (CN) phase	184.00
601SC	Polar (NH ₂) phase	184.00

PRE-COLUMN KITS

Catalog No.	Material	Price
101PSC	Silica adsorbent	\$ 76.00
201PSC	Reverse phase (C ₁₈)	92.00
301PSC	Anion Exchange	102.00
401PSC	Cation exchange	102.00
501PSC	Polar (CN) phase	92.00
601PSC	Polar (NH ₂) phase	92.00

Pre-column kits contain the necessary hardware to make a guard column and seven grams of the designated solid core material, sufficient to fill the guard column three times.

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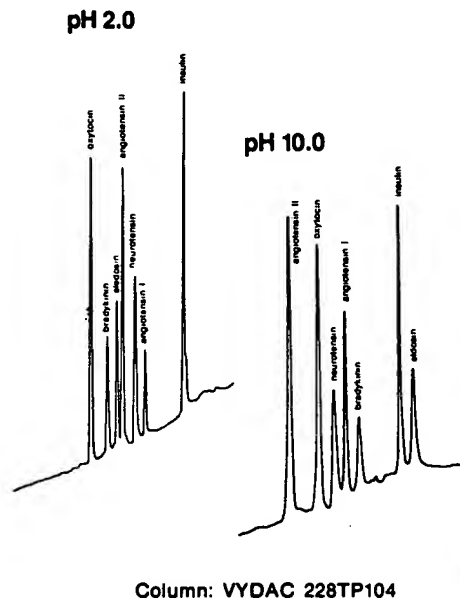
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NEW HPLC Products from VYDAC!

VYDAC pH Stable Reverse Phase Columns are C-8 columns based on VYDAC wide pore TP silica and are stable from pH 2 to 10. pH stable reverse phase columns are ideal for chromatographing biomolecules at neutral or basic pH and add a new dimension to the separation of peptides.

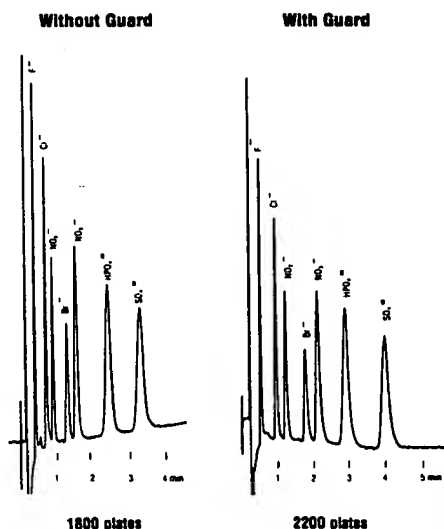
AFFECT OF pH ON RELATIVE RETENTION OF PEPTIDES



VYDAC High Performance Guard Columns are packed with the same material as analytical columns. High Performance Guard Columns fit directly on top of VYDAC analytical columns and can actually enhance performance while increasing column lifetime.

AFFECT OF GUARD COLUMN ON ANALYTICAL COLUMN PERFORMANCE

Column: VYDAC 300IC405

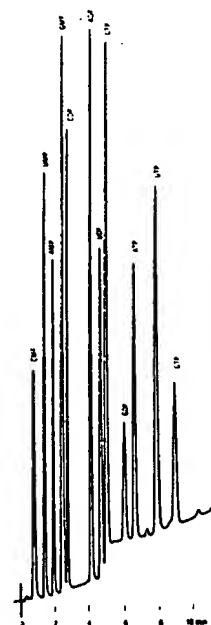


The VYDAC Nucleotide Analysis Column can separate the twelve major nucleotides in 10 minutes.

SEPARATION OF MAJOR NUCLEOTIDES

Column: VYDAC 303NT405

- 1 - CMP
- 2 - UMP
- 3 - AMP
- 4 - GMP
- 5 - CDP
- 6 - ADP
- 7 - UDP
- 8 - CTP
- 9 - GDP
- 10 - ATP
- 11 - UTP
- 12 - GTP



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